Natural Resources for Human Health
eISSN: 2583-1194

Original Research

Received 28 May 2026
Revised 24 June 2026
Accepted 20 July 2026

Natural Resources for Human Health 2026, Vol. 6(9s): 1317–1330

KEYWORDS:
Biological control, Antagonistic activity, Systemic resistance

Biocontrol of White Mold caused by Sclerotinia Sclerotiorum in Cucumber using Trichoderma Aggressivum, Bacillus Paralicheniformis, and Earthworms: First Study in Iraq

Ahmed S. Hasan[1], Assist. Prof. Dareen S. Jameel[2]

[1,2] Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad, Iraq
[1] ahmed.sabah2204m@coagri.uobaghdad.edu.iq
[2] dareen.safwat@coagri.uobaghdad.edu.iq

ABSTRACT: Sclerotinia sclerotiorum causes white mold in crops, leading to severe yield losses in cucumber and many vegetables. This study evaluated the biocontrol potential of the Trichoderma aggressivum, Bacillus paralicheniformis, and earthworms Aporrectodea trapezoides against this pathogen. The pathogen was isolated from infected plants and evaluated for pathogenicity in pots experiments. In vitro, T. aggressivum showed strong antagonism (Bell's scale grade 2), B. paralicheniformis inhibited fungal growth by 89.7% and earthworms consumed 80% of hydrated sclerotia. Under plastic house conditions, all treatments reduced disease incidence (T. aggressivum: 76.67%, B. paralicheniformis: 83.33%, Earthworms: 86.67%) and severity (AUDPC: 82.2, 99.03, 116.48 respectively). They also increased peroxidase activity (79.77, 65.8, 62.03 ΔAbs/min/g) and phenolic content (118.1, 101.5, 96.5 mg/g).

INTRODUCTION

Sclerotinia sclerotiorum is a plant pathogen that infects over a few hundred monocotyledonous and dicotyledonous plant species, including economically important crops like cucumbers, tomatoes, soybeans, and edible dry beans, which causes significant yield losses (17). This fungus causes white mold disease, which leads to soft rot and fast decay of plant tissue, often resulting in major yield loss (14). Chemical fungicides are commonly used to control soil-borne diseases such as *S. sclerotiorum*. However, their excessive use can pose health risks, leave chemical residues in crops, harm beneficial soil microorganisms, promote the development of resistant fungal strains, and negatively impact the environment and soil health, including the earthworms that inhabit it (3, 31). As an alternative, biological control strategies have gained more attention as they can be a practical alternative for managing plant diseases. Plant Growth-Promoting Rhizobacteria (PGPR) have been successfully used to reduce soilborne diseases and support plant growth (2, 28). *Bacillus* species are among the PGPR known for their strong ability to combat plant fungal diseases and serve as effective biocontrol agents against plant pathogens (5). These bacteria contribute to overall plant health by suppressing fungal growth and enhancing nutrient availability (20). *Bacillus paralicheniformis* has demonstrated significant biocontrol potential due to its ability to produce bioactive secondary metabolites, including fengycin, bacillibactin, and bacitracin, which contribute to its antagonistic activity against plant pathogens (8). *Trichoderma spp.* can inhibit soil-borne pathogens like *S. sclerotiorum* through mechanisms like mycoparasitism, production of antibiotics, and promoting plant growth, which enhances the plant's defense mechanisms and induce systemic resistance against pathogens. furthermore, the application of *Trichoderma spp.* significantly improves vegetative growth and yield traits in crops such as potato, as it improves some parameters including increased stem number, leaf area, chlorophyll content, tuber production, and carbohydrate percentage (33, 1). *Trichoderma aggressivum* f. *sp. europaeum* has been shown to reduce disease severity caused by *Pythium ultimum* by nearly 63%, while also improving root growth under salt stress, confirming its potential as both a biocontrol agent and plant growth

promoter (32). Earthworms not only improve soil health and microbial diversity but also play an important role in suppressing soilborne diseases. Their presence has been associated with enhanced plant growth and reduced disease severity in crops like tomato, eggplant, and asparagus, likely through the shift of beneficial microbial populations in the rhizosphere. Furthermore, The decomposition products of earthworms are rich in nutrients and growth-promoting compounds that support microbial growth, highlighting their potential role in enhancing soil fertility and serving as natural biofertilizers which can inhance plant growth and resistance against pathogens (11, 37). Recent findings also confirm that earthworms significantly influence bacterial community structure, decomposition rates, and root development which supports their potential as a sustainable addition for soil and plant health management (35). This study aimed to evaluate the efficacy of various biological agents in controlling white mold disease caused by *S. sclerotiorum* on cucumber. The research included isolation and identification of the pathogen, assessment of the pathogenicity of selected isolates on cucumber seedlings under pot conditions, and in vitro evaluation of the antagonistic activity of *T. aggressivum* and *B. paralicheniformis*. Additionally, the ability of earthworms (*Aporrectodea trapezoides*) to consume *S. sclerotiorum* sclerotia was examined under laboratory conditions. The study also investigated the individual effects of *B. paralicheniformis*, earthworms, and *T. aggressivum* on disease incidence, area under the disease progress curve (AUDPC), peroxidase activity, and total phenol content in cucumber plants, in order to evaluate their role in inducing systemic resistance in the field under plastic house conditions.

MATERIALS AND METHODS

Isolation of the Pathogenic Fungus *Sclerotinia sclerotiorum*: Infected samples of cucumbers showing symptoms of *S. sclerotiorum* infection were randomly collected from plastic houses during December of the 2023–2024 growing season. Samples were taken from 3 locations: Al-Yusufiyah,Wasit, and Al-Jadriya (University of Baghdad). The infected plants exhibited characteristic symptoms such as white mycelial growth and the presence of sclerotia. The samples were placed in polyethylene bags labeled with collection site and date, and transported to the Plant Pathology Laboratory, Department of Plant Protection, College of Agricultural Engineering Sciences for further examination. Sclerotia were individually collected from infected plant stems. Surface sterilization was carried out using a 1% sodium hypochlorite solution for 2 minutes, followed by rinsing in sterile distilled water for 1 minute and drying on filter paper. Each sclerotium was cultured on a 9 cm Petri dish containing sterile PDA medium, which had been autoclaved at 121°C and 1.5 kg/cm² pressure for 15 minutes. Cephalexin antibiotic was added to the medium at a concentration of 250 mg/L. The inoculated plates were incubated at 25±2°C for five days until fungal mycelium developed. Each isolate was purified separately by transferring a portion of the actively growing mycelial edge to new sterile PDA plates, which were then incubated at 25±2°C until sclerotia formation occurred.

Pathogenicity Test of *Sclerotinia sclerotiorum* Isolates on Cucumber seedlings in Pots: This experiment was conducted at Research Station B, Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad. Three fungal isolates of *S. sclerotiorum* were tested on cucumber plants in pots. A sterilized loamy soil was prepared using an autoclave at 121°C and 1.5 kg/cm² pressure for one hour, repeated twice. The soil was placed in plastic pots, each containing 2 kg of soil. Cucumber seedlings (15 days old) were transplanted into the pots, with three seedlings per pot and three replicates for each fungal isolate. Inoculation followed the method of Zhang *et al.* (38). Wooden toothpicks, 3 cm in length and of uniform thickness, were soaked in 5% sugar solution in glass flasks and sterilized in an autoclave at 121°C and 1.5 kg/cm² pressure for 20 minutes. The sterilized toothpicks were then placed on sterile PDA medium in Petri dishes and inoculated with a 5 mm mycelial disc from each fungal isolate separately. The dishes were incubated for two weeks to allow fungal colonization. Prior to inoculation, cucumber plants were misted with sterile distilled water. Inoculated toothpicks were inserted into the main stem near the shoot apex of each plant. The pots were then covered with polyethylene bags for three days to maintain humidity. Three non-inoculated pots served as controls for comparison. Plants were irrigated as needed and monitored. Data were recorded 15 days post-inoculation. Koch's postulates were fulfilled by re-isolating the fungus from the infection sites and culturing it on PDA medium to confirm the presence of *S. sclerotiorum*. The percentage of infected plants was calculated using the following formula:

$$\%\text{incidence} = \frac{\text{number of infected plants}}{\text{Total number of plants}} \times 100 \quad (1)$$

Disease severity was assessed using the disease rating scale by Dixon and Doodson (10), which includes six levels:

0= No visible symptoms

1=Lesion length does not exceed 2 cm.

3=Lesion length more than 2 and up to 4 cm.

4= Lesion length more than 6 cm and up to 8 cm, without complete stem girdling. 5=Lesion length exceeds 8 cm with complete stem girdling, causing wilting and plant death. The disease incidence and infiction severity was calculated according to Mickenny's equation (27). The most virulent isolate was identified on the molecular level using PCR method and confirmed that the isolate belongs to *Sclerotinia sclerotiorum* and was registered in GenBank and assigned the accession number PV082170.

Antagonistic Activity Test of *Trichoderma aggressivum* Against the Pathogenic Fungus *Sclerotinia sclerotiorum*: The dual culture technique was employed to assess the antagonistic interaction between the biocontrol fungus *T. aggressivum* (obtained from the Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad) and the pathogenic fungus *S. sclerotiorum*. A 5 mm mycelial disc of *T. aggressivum*, aged five days, was placed at the center of one half of a Petri dish containing PDA medium, while an identical disc from a five-day-old colony of *S. sclerotiorum* was placed at the center of the other half using a sterile cork borer. Control plates containing a single disc of either fungus were also prepared. The dishes were incubated at a temperature of 25 ± 2°C (16). The experiment was conducted with four replicates, and observations were recorded after one and two weeks of incubation. The antagonistic effect was evaluated according to the scale developed by Bell *et al.* (6), which consists of five grades:

Grade 1: The biocontrol fungus completely overgrows the pathogenic fungus.

Grade 2: The biocontrol fungus covers three-quarters of the Petri dish.

Grade 3: Each fungus occupies half of the dish.

Grade 4: The pathogenic fungus covers three-quarters of the dish.

Grade 5: The pathogenic fungus fully covers the dish.

*The biocontrol fungus is considered effective when it exhibits grade 1 or grade 2 antagonism.

Antagonistic Activity Test of *Bacillus paralicheniformis* Against the Pathogenic Fungus *Sclerotinia sclerotiorum*: The antagonistic activity of *B. paralicheniformis* against *S. sclerotiorum* was evaluated using the dual culture technique (the bacterium was previously obtained from soil and molecularly identified as *B. paralicheniformis* and was registered in GenBank and assigned the accession number PV089010) . A 5 mm plug from a 5-day-old culture of the pathogen was placed on one side of a PDA plate. On the opposite side, *B. paralicheniformis*, pre-cultured for two days on nutrient agar (NA), was streaked using a sterile loop. The plates were incubated at 25 ± 2°C for seven days, after which the growth of fungal mycelium was assessed. Control plates were inoculated with the pathogen alone. The zone of inhibition between the pathogen and the bacterial isolate was measured to determine antagonistic activity. The experiment followed a completely randomized design (CRD) with four replications. The percentage of radial growth inhibition was calculated using the following formula:

$$\mathbf{I\%} = \frac{\mathbf{R1 - R2}}{\mathbf{R1}} \times \mathbf{100} \qquad (\mathbf{2})$$

Where:

I% = inhibition Percentage of radial growth

R1 = Maximum radius of mycelial growth on the control plate

R2 = Maximum radius of mycelial growth toward the bacterial colony on the treated plate.

Determining the Most Effective Dilution of *Bacillus paralicheniformis* Against *Sclerotinia* sclerotiorum: To find the most effective concentration of *B. paralicheniformis* in reducing the growth of *S. sclerotiorum*, serial dilutions were prepared from a 48-hour-old culture grown in Nutrient Broth. One milliliter of the culture was added to 9 mL of sterile distilled water to create dilutions ranging from 10^{-1} to 10^{-8}. From each dilution, 1 mL was mixed into melted Potato Dextrose Agar (PDA) before it solidified. The plates were gently swirled to distribute the bacteria evenly. Three replicate plates were prepared for each dilution, along with three control plates containing only PDA. Once the medium had solidified, a 5 mm plug was taken from the edge of a 5-day-old *S. sclerotiorum* culture and placed at the center of each plate. Plates were incubated at 25 ± 2°C for three days. After incubation, fungal growth was measured, and the percentage of inhibition was calculated using the equation

$$(3) \quad \textbf{PIRG \%} = \frac{\textbf{R1} - \textbf{R2}}{\textbf{R1}} \times \textbf{100}$$

where:
PIRG = Percentage inhibition of radial growth

R1= the fungal radial growth (in cm) on control plates without the bacterial treatment
R2= the radial growth (in cm) of *S. sclerotiorum* on plates treated with the bacterial isolate.

Evaluation of Earthworm Activity in the Sclerotia Consumption of *Sclerotinia sclerotiorum* in vitro: An in-vitro experiment was conducted to evaluate the ability of the earthworm *A. trapezoides* to consume hydrated sclerotia of the pathogenic fungus *S. sclerotiorum*, in order to investigate its potential impact on the pathogen's survival structures. The sclerotia were soaked in sterile distilled water for 13 weeks to ensure complete hydration and readiness for consumption by the earthworms (12). Plastic containers measuring 30×25 cm were used, each filled with 1 kg of sterilized soil at a depth of 5 cm. The containers were perforated at the top to allow air exchange for the worms. Ten earthworms were introduced into each container and allowed to acclimate to the soil for three days under suitable environmental conditions (temperature 20–25°C, adequate moisture, and complete darkness). After the acclimation period, 20 pre-weighed hydrated sclerotia were evenly distributed into the soil of each container. The experiment lasted for 15 days, during which the containers were maintained under stable conditions to allow worm mobility and sclerotia consumption, with daily monitoring and soil moistening. At the end of the experiment, the remaining sclerotia were separated from the soil using a fine sieve to ensure complete recovery. The recovered sclerotia were classified into three categories:

1- Intact: Undamaged, showing no signs of consumption.

2- Partially consumed: Showing signs of damage and partial mass loss.

3- Fully consumed: Not found in the container, indicating complete consumption by the earthworms.

The number and weight of sclerotia were measured before and after the experiment using a precision balance. The percentage of consumed sclerotia was calculated using the following formula:

$$\textbf{(2). Percentage of consumed sclerotia} = \left(\frac{(\textbf{Initial number} - \textbf{Recovered number})}{\textbf{Initial number}}\right) \times \textbf{100}$$

Likewise, the consumed weight of the sclerotia was calculated using the formula:

$$\textbf{(3). Consumed weight percentage} = \left(\frac{(\textbf{IInitial weight} - \textbf{Final weight})}{\textbf{Initial weight}}\right) \times \textbf{100}$$

The effect of *Trichoderma aggressivum*, Earthworms, and *Bacillus paralicheniformis* on Disease incidence Severity, and Systemic Resistance Induction Against *Sclerotinia sclerotiorum* in Cucumber Under Plastic House Conditions: The experiment was conducted in a plastic house at Research Station B, Department of Plant Protection, College of Agricultural Engineering Sciences, University of Baghdad, during the 2024-2025 growing season. The soil was prepared by performing all necessary agricultural operations, including weed removal, plowing, leveling, and smoothing. Afterwards, the soil was sterilized using the fungicide Beltanol and left for one month to ensure the elimination of any pesticide residues. The greenhouse was divided into five beds, each measuring 25 meters in length and 80 cm in width per plot, with a one-meter distance between plots. A drip irrigation system was installed to provide optimal conditions for the experiment. Treatments were arranged in a randomized design with three replicates per treatment, each replicate containing 10 plants, along with a control treatment (without any additions). Fifteen-day-old cucumber seedlings were planted with a spacing of 40 cm between plants. After the plants had settled for several days, the biocontrol fungus *T. aggressivum* was inoculated on *Panicum miliaceum* seeds and applied to the soil at a rate of 1 gram per plant at a depth of 5 cm for the treatments containing the biocontrol fungus. the inoculated seeds were prepared by first washing and sterilizing the seeds in flasks using an autoclave at 121°C and 1.5 kg/cm² pressure for 15 minutes on two consecutive occasions. Then, each flask was inoculated with five 0.5 cm diameter agar discs taken from the actively growing edges of 7-day-old *T. aggressivum* cultures on PDA medium. The flasks were incubated at 25 ± 2°C for two weeks with continuous shaking to ensure even fungal colonization before being mixed with the soil. The bacterium *B. paralicheniformis* was applied by irrigating the root zone of the plant with 10 mL of the effective dilution (10^{-5}) for the treatments containing the bacterium. Additionally, earthworms (3 worms per plant) were introduced into the soil around the roots for the

treatments containing earthworms. A control treatment was also included, consisting only of cucumber plants inoculated with *S. sclerotiorum* (SC1 isolate) without the addition of any biocontrol agents and was replicated three times. Leaf samples were collected from plants treated with the mentioned factors for peroxidase enzyme analysis, which was conducted following the method described by Hammerschmidt *et al.* (15), and phenol content analysis according to the method of Meena *et al.* (27), at 7, 14, and 21 days after the fungal inoculation. The incidence was calculated after one week using equation (1), and disease severity was estimated over three weeks based on the disease rating scale of Dixon and Doodson (10). Disease severity was calculated according to McKinney's formula (26). The Area Under Disease Progress Curve (AUDPC) was also calculated according to the formula by Cooke *et al.* (9) :

AUDPC= ½ ∑(2S1+2S2+⋯+SX)

where:

S1= first severity reading

S2= second severity reading

SX= last recorded severity reading

RESULTS AND DISCUSSION

Isolation of the Pathogenic Fungus *Sclerotinia sclerotiorum*: Three isolates of the pathogenic fungus *S. sclerotiorum* were obtained from infected cucumber plants grown in plastic houses (Table1). Four to five days after inoculating the plates, the fungal colonies showed white growth on the PDA medium, with light aerial mycelium extending vertically from the surface of the plate to the lid. After approximately eight days, white aggregations formed due to mycelial accumulation, initially appearing as

Table1. Fungal isolates and their location

isolate name	sampling location
SC1	Al-Yusufiyah
SC2	Al-Jadriya
SC3	Wasit

white masses accompanied by transparent exudates on the surface of the isolates. These gradually turned black, forming hard sclerotia within 8–10 days after inoculation (Figure1). Differences were observed among the isolates in the shape, size, number, and distribution of the sclerotia within the plate (Figure2). This variation may be attributed to morphological diversity, differences in growth rate and sclerotia production, as well as genetic and environmental variation among the isolates (18).

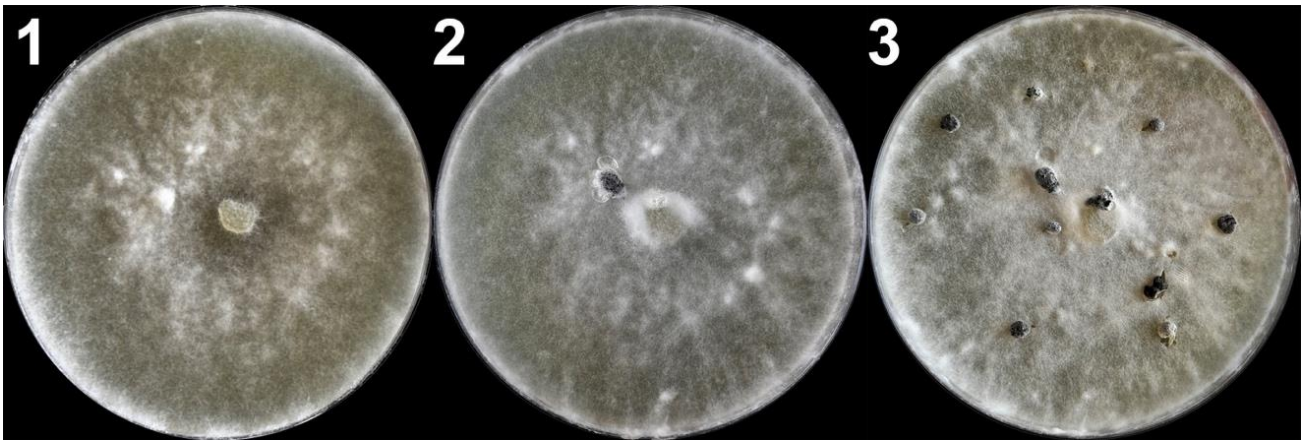


Figure 1. Sclerotia development stages on PDA: 1) White mycelial colony at 5 days; 2) Hyphal aggregation into white masses (8–10 days); 3) Mature black sclerotia formed (15–20 days).

Natural Resources for Human Health
eISSN: 2583-1194

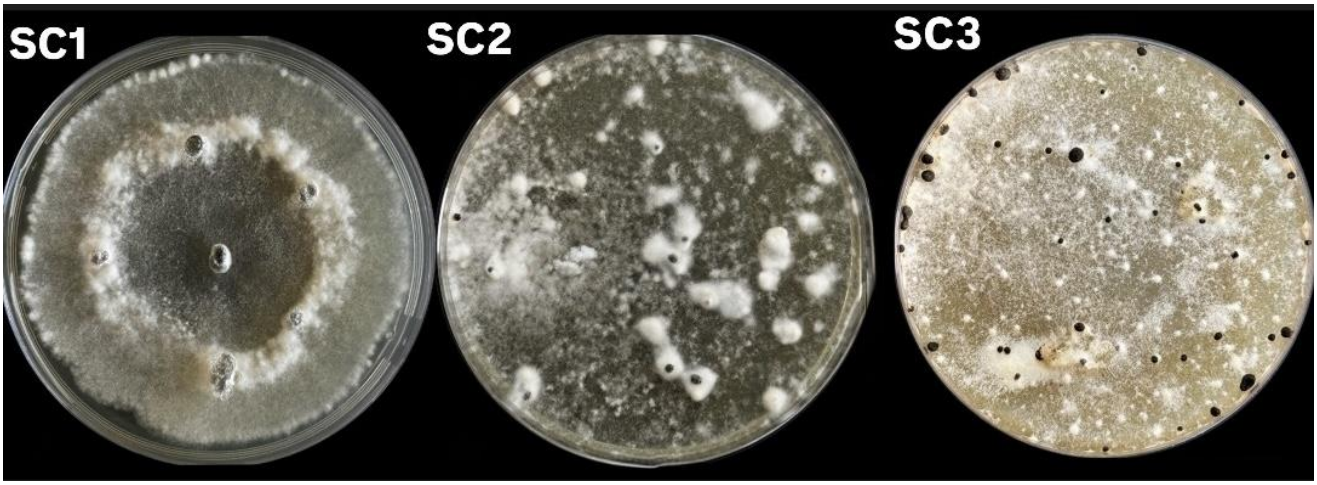


Figure 2: Different Distribution of sclerotia on PDA medium among *S. sclerotiorum* isolates at an age ranging from 15-20 days.

Pathogenicity Test of *Sclerotinia sclerotiorum* Isolates on Cucumber seedlings in Pots: The results of the pathogenicity test in pots (Table 2, Figure 3) showed that all isolates were pathogenic to cucumber seedlings, with significant differences in incidence and severity. Isolate SC1 recorded the highest incidence at 100%, which was significantly different from the control treatment (0%), but not significantly different from isolate SC2, which had an incidence of 88.9%. This was followed by isolate SC3, which showed an incidence of 66.6% and differed significantly from the other isolates. Isolate SC1 also showed the highest severity of infection at 90.7%, not significantly different from SC2, which recorded 73.0%. Isolate SC3 showed an infection severity of 44.0%, which was significantly different from the other isolates, while the severity in the control treatment was 0%. The variation in disease

incidence and severity may be attributed to genetic differences among the fungal isolates and their ability to cause disease, as well as the influence of the host plant from which these isolates were obtained.

these results were consistent with Buchwaldt (7) as he indicated that infection severity varies among *S. sclerotiorum* isolates, where genetic differences among isolates affected disease severity in canola (Brassica napus).

Table 2. Pathogenicity test of the isolated pathogen *S.sclerotiorum* on cucumber seedlings.

isolate name	% incidence	% of disease severity
SC1	100	90.7
SC2	88.9	73.0
SC3	66.6	44.0
Control	0.0	0.0
LSD(0.05)	19.26	19.63

Natural Resources for Human Health
eISSN: 2583-1194

Figure 3. Symptoms and severity of disease on cucumber seedlings in pots, showing the effects of different isolates of the pathogenic fungus *S. sclerotiorum* (isolate names labeled on each image). The control treatment is displayed on the far left.

Antagonistic Activity Test of *Trichoderma aggressivum* Against the Pathogenic Fungus *Sclerotinia sclerotiorum:*

The results showed that the biocontrol fungus *T. aggressivum* exhibited a high antagonistic activity against the pathogenic fungus *S. sclerotiorum*, recording an antagonism rating of 2 according to the scale of Bell *et al.* (6) , which represents the highest level on the scale, after seven days of inoculating the culture medium (Figure 4). It was also observed that the biocontrol fungus made contact with the mycelium of the pathogenic fungus, followed by the overgrowth of its colony over the pathogen's mycelium The antagonistic ability of *T. aggressivum* is attributed to several factors that make it an effective biocontrol agent against various plant-pathogenic fungi. Among these factors is its ability to directly parasitize the pathogen's mycelium by coiling around its hyphae and degrading its cell walls using hydrolytic enzymes it secretes. Furthermore, it is characterized by its rapid growth and competitive ability for nutrients and space, along with the production of growth-inhibiting compounds such as antibiotics and hydrolytic enzymes, including β-1,3-glucanases and chitinases. These traits are considered key mechanisms by which *Trichoderma spp.* suppress plant pathogens (13, 4). This finding aligns with several previous studies (25, 24).

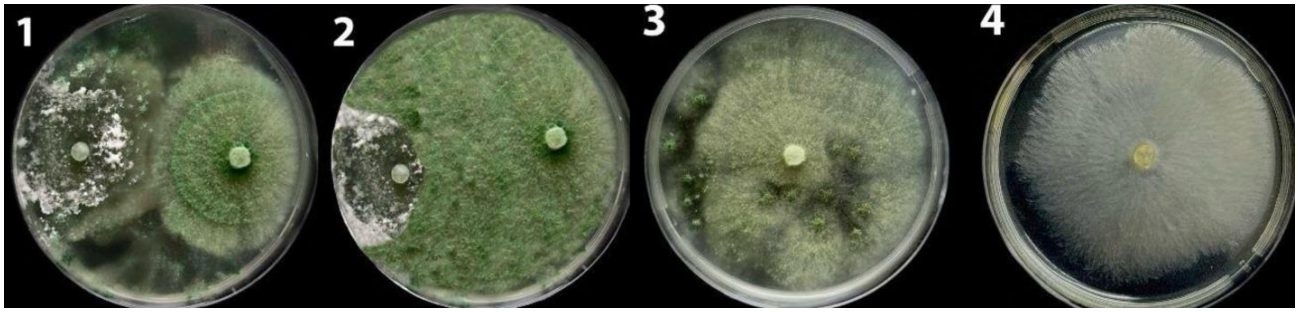


Figure 4. Antagonism of *T. aggressivum* against *S. sclerotiorum*
1: After 1 week; 2: After 2 weeks; 3: *T. aggressivum* alone; 4: *S. sclerotiorum* alone.

Antagonistic Activity Test of *Bacillus paralicheniformis* Against the Pathogenic Fungus *Sclerotinia sclerotiorum*: The results of this test showed that using *B. paralicheniformis* as a biological control agent led to a significant inhibition of *S. sclerotiorum* growth. Using the dual culture technique on PDA medium, the inhibition percentage reached 89.7% after 7 days of incubation, compared to the control treatment which showed 0% inhibition (Table 4, Figure 5). The biocontrol potential of *B. paralicheniformis* against phytopathogenic fungi has been linked to its ability to produce antifungal metabolites such as lichenysin, bacillibactin, and fengycin. Its antagonistic properties align with earlier studies showing that various *Bacillus* species isolated from

plant surfaces and rhizospheres can suppress pathogens like *Sclerotinia sclerotiorum* on wheat and barley. These studies demonstrated the effectiveness of several

Bacillus strains in inhibiting fungal growth through bioactive compounds. Similarly, *B. paralicheniformis* was identified as a potent biocontrol agent with strong antifungal activity against pathogens such as *Fusarium oxysporum*, *Curvularia microspora*, *C. akaii*, and *Rhizoctonia solani* (36, 21).

Table 4. Antagonistic Effect of *B.paralicheniformis* on *S. sclerotiorum*

treatments	inhibition%
B. paralicheniformis	**89.7**
Control	**0.00**
LSD 0.05	**8.97**

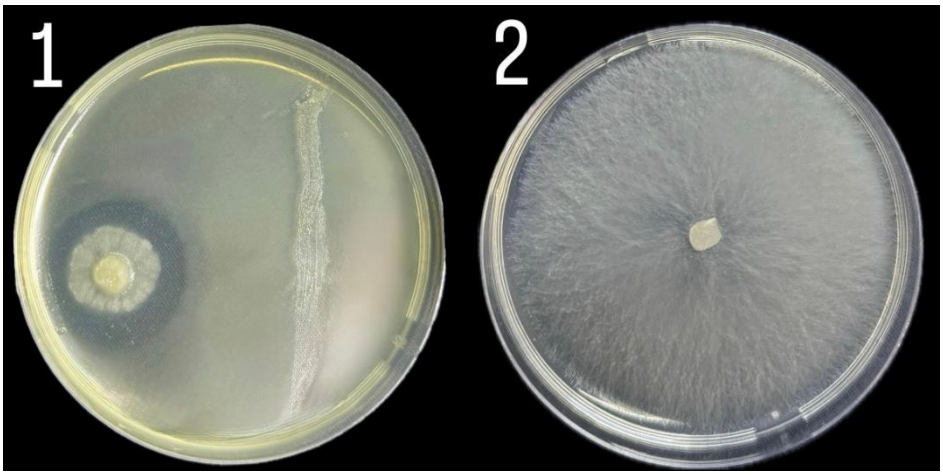


Figure 5. Antagonistic activity of *B. paralicheniformis* against *S. sclerotiorum* on PDA after 7 days of incubation. 1: Interaction showing inhibition zone; 2: Control plate with *S. sclerotiorum* alone.

Determining the Most Effective Dilution of *Bacillus paralicheniformis* Against *Sclerotinia* sclerotiorum: The results showed that the bacterial suspension was able to suppress the growth of *Sclerotinia sclerotiorum* effectively up to the 10^{-5} dilution. At this level, a noticeable reduction in fungal growth was observed compared to the untreated control.

Evaluation of Earthworm Activity in Consuming the Sclerotia of *Sclerotinia sclerotiorum* under Laboratory Conditions:

The results in (Table 3) showed that earthworms *A. trapezoides* demonstrated a significant ability to consume the sclerotia of the pathogenic fungus *S. sclerotiorum* after sufficient hydration, with a significant difference compared to the control treatment. The percentage of consumed sclerotia by number reached 80%, while the consumed weight percentage reached 75.9%, compared to the control treatment which recorded no consumption in either number or weight. This ability is attributed to the feeding behavior and digestive system of earthworms, as they can feed on sclerotia and break down their structure, thereby reducing the survival of the fungus in the soil and decreasing the sources of primary infection. These results indicate that earthworms may play a promising role as a biological control agent by helping to reduce the quantity of *S. sclerotiorum* sclerotia in the soil. These findings are consistent with Euteneuer *et al.* (12), who reported that earthworms consumed up to 61.1 ± 26.0% of sclerotia, contributing to their reduction through ingestion.

Table 3. Percentage of Sclerotia Number and Weight Consumed by Earthworms

treatment	% of consumed sclerotia	%of Consumed weight
Earthworms	**80**	**75.9**
Control	**0.0**	**0.0**
LSD 0.05	**12.42**	**19.40**

Effect of *Trichoderma aggressivum*, Earthworms, and *Bacillus paralicheniformis* on incidence, Severity, and Systemic Resistance Induction Against *S. sclerotiorum* in Cucumber Under Plastic House Conditions: The results in (Table 4) showed significant differences in disease incidence among the biocontrol treatments compared to the control. The treatment involving *T. aggressivum* + SC1 recorded the highest significant reduction in disease incidence, reaching 76.67%, and differed significantly from the treatments *Bacillus paralicheniformis* + SC1 and Earthworms + SC1, which recorded reductions of 83.33% and 86.67%, respectively, with no significant difference between them. All treatments showed statistically significant reductions compared to the control treatment (SC1 isolate without any addition), which recorded a disease incidence of 96.67%.

Table 4. Effect of biocontrol agents on disease incidence in cucumber plants under plastic house conditions

Treatment	disease incidence
***T. aggressivum* +SC1**	**76.67**
***B. paralicheniformis* + SC1**	**83.33**
Earthworms + SC1	**86.67**
Control (SC1)	**96.67**
LSD 0.05	**7.63**

The results in (Figure 6) also showed significant differences in disease severity by lowering the area under disease progress curve (AUDPC) among the biocontrol treatments compared to the control. The treatment *T. aggressivum* + SC1 recorded the lowest value of 82.20, followed by the treatment *B. paralicheniformis* + SC1, which recorded an AUDPC value of 99.03. Followed by Earthworms + SC1 treatment, with an AUDPC value of 116.48, while the control treatment (SC1 without any addition) recorded the highest AUDPC value of 158.32.

The results in (Figure 7) also showed that all treatments significantly increased peroxidase activity in cucumber plants infected with the pathogen in comparison to the control treatment (SC1 without any addition). The enzyme activity was measured based on the rate of change in absorbance per minute per gram of fresh weight. Peroxidase levels began to rise on day 7, peaked at day 14, and then gradually declined. The *T. aggressivum* + SC1 treatment recorded the highest significant increase, reaching 56 and 79.77 at one and two weeks after adding the treatment, respectively. It was followed by the *B. paralicheniformis* + SC1 and Earthworms + SC1 treatments, which recorded 46.27 and 65.8, and 45.93 and 62.03, respectively without a significant difference between them. In contrast, the control treatment showed lower enzyme levels of 38.17 and 47.53 respectively.

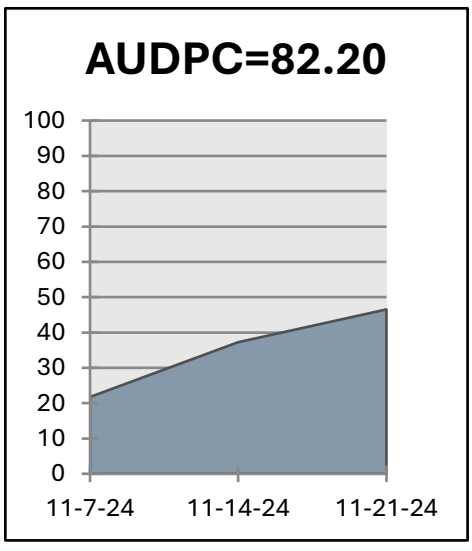
the results in (Figure 8) also showed that all treatments led to a significant increase in total phenolic content compared to the control treatment. Phenolic content was measured in milligrams per gram of fresh plant tissue. Levels began to rise on day 7, peaked on day 14, then gradually declined. The highest increase was recorded in the *T. aggressivum* + SC1 treatment, reaching 86.6 and 118.1 at one and two weeks after treatment, respectively. This was followed by the *B. paralicheniformis* + SC1 and Earthworms + SC1 treatments, which recorded 82.27 and 101.5, and 79.13 and 96.5 respectively with no significant difference between them. The control treatment recorded the lowest values, 65.77 and 85.3 respectively.

The biological fungus *Trichoderma* spp. is considered one of the most effective biocontrol agents used against a wide range of plant pathogens. This effectiveness is related to its multiple defense mechanisms, which include competing with pathogens for nutrients and space, producing antifungal compounds such as peptaibols, gliotoxin, and trichokonins, and breaking down fungal

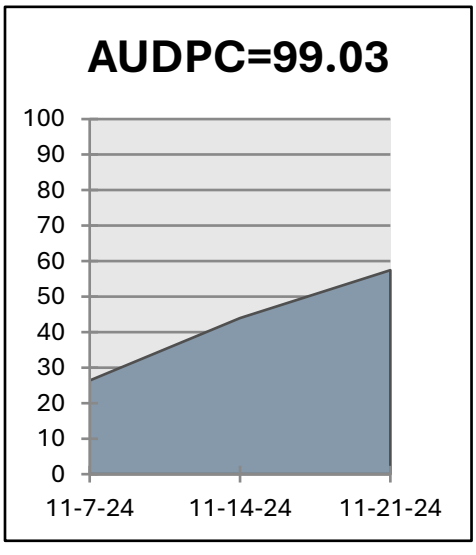
cell walls through enzymes like chitinases and β-1,3-glucanase. One of its most important roles is inducing systemic resistance in plants. *Trichoderma* releases natural elicitors such as chitin oligosaccharides and β-glucans that stimulate the plant's defense systems. This enhances the plant's ability to resist pathogens, even without direct contact between the fungus and the pathogen (29). These findings are consistent with Singh *et al.* (34), who demonstrated that the induction of resistance against pathogens is closely linked to the ability of *Trichoderma* spp. to colonize plant roots. This root colonization is considered one of the key mechanisms in protecting plants from disease. Their study showed that eggplant plants treated with the biocontrol fungi *Trichoderma harzianum* and *Trichoderma asperellum*, and infected with *S. sclerotiorum*, exhibited an increase in total phenolic compound production 72 hours after infection. Additionally, there was a recorded rise in shikimic acid levels in the treated plants. The study also reported a notable increase in the activity of plant defense enzymes such as Phenylalanine Ammonia Lyase (PAL), peroxidase, and Polyphenol Oxidase (PPO) compared to other treatments. These results highlight the ability of *Trichoderma* spp. to reprogram the plant's defense system and provide effective protection against pathogenic fungi.

Bacillus spp. play a vital role in activating plant defense mechanisms, including induced systemic resistance (ISR), through the production of elicitor compounds. These compounds trigger the jasmonic acid signaling pathway in the host plant, which in turn strengthens the plant's chemical and physical barriers against pathogen invasion which could be a possible mechanism in reducing disease incidence and severity (22). The results were consistent with (19) that highlight the role of *B. paralicheniformis* in activating induced systemic resistance (ISR) in plants. This mechanism is reflected in the increased activity of key defense enzymes such as peroxidase, superoxide dismutase, phenylalanine ammonia lyase, and polyphenol oxidase, along with elevated proline levels. These responses contribute to enhanced antioxidant defense and structural resistance which supports plant health by priming internal defense pathways in addition to the antagonistic activity of this bacteria.

the presence of earthworms can induce systemic resistance (ISR) in plants by modulating gene expression related to both biotic and abiotic stress responses. These responses were found to resemble those triggered by chemical signals released by plant growth-promoting rhizobacteria (PGPR), suggesting that earthworms may release signaling molecules such as auxin and ethylene. These molecules act as elicitors, priming the plant's defense system and enhancing its ability to resist pathogens even before infection occurs, which strengthening the plant's natural defenses (30). Earthworms contribute to induced systemic resistance (ISR) by enhancing plant immunity through their activity in the soil. Their presence and their casting increases the activity of defense enzymes like polyphenol oxidase, peroxidase, and phenylalanine ammonia lyase, which strengthen plant defenses against pathogens (23).



***T. aggressivum* + SC1**



***B. paralicheniformis* + SC1**

Natural Resources for Human Health
eISSN: 2583-1194

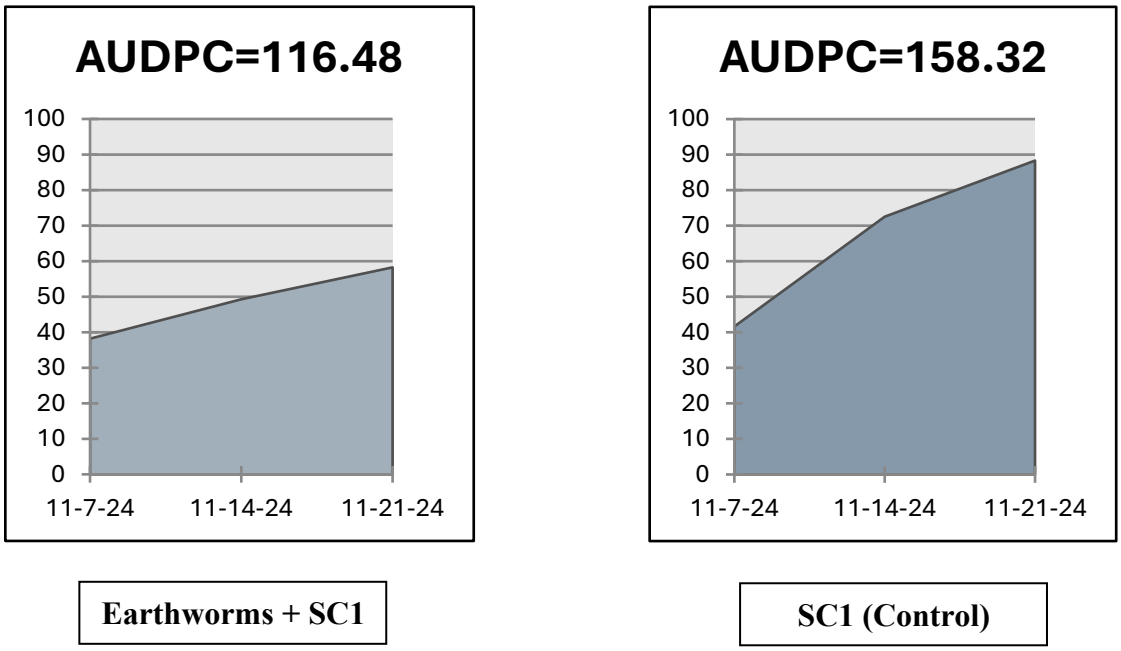


Figure 6. Area Under the Disease Progress Curve (AUDPC) of the pathogenic fungus *S. sclerotiorum* on cucumber plants under plastic house conditions. The x-axis represents the date of disease severity readings, and the y-axis represents the disease severity values.

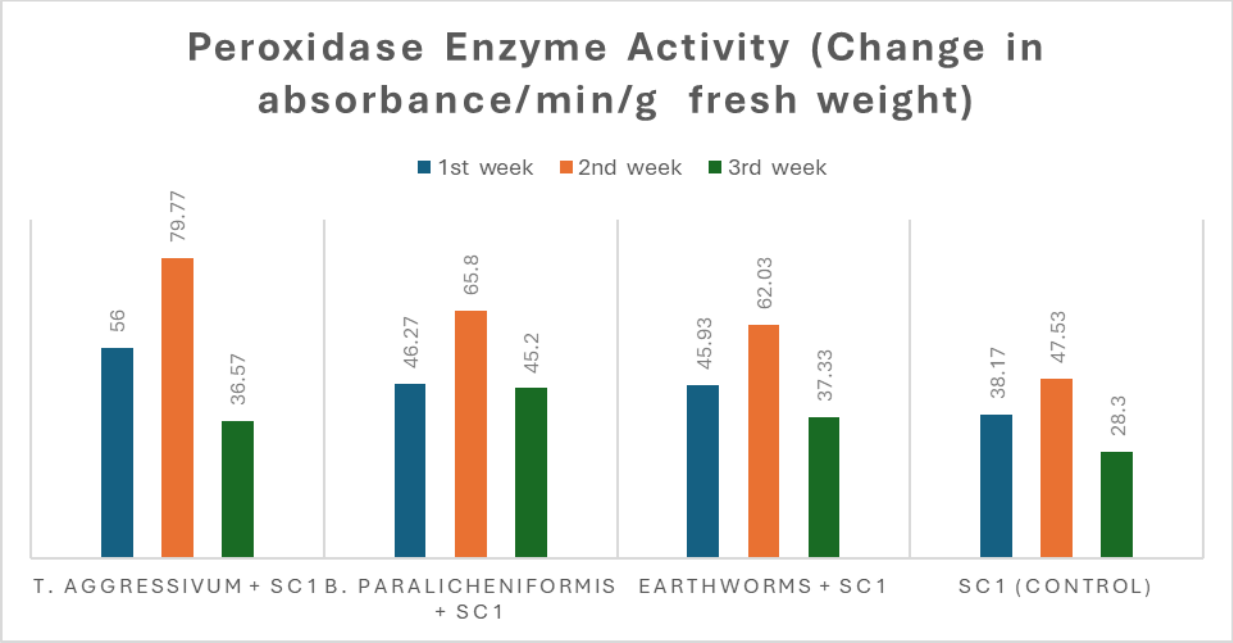


Figure 7. Effect of biocontrol agents on peroxidase activity (ΔAbs/min/g fresh weight) in cucumber plants infected with *S. sclerotiorum* under plastic house conditions.
Values represent means of 3 replicates. LSD (0.05): 7 days = 5.80, 14 days = 5.16, 21 days = 5.68.

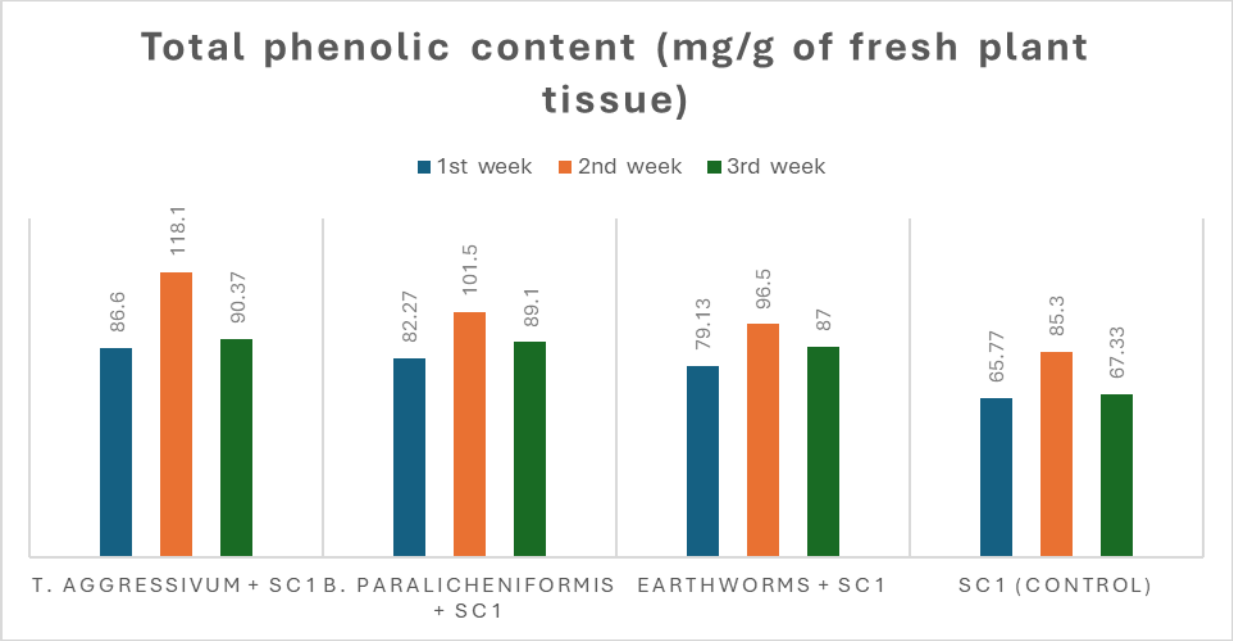


Figure 8. Effect of biocontrol agents on total phenolic content (mg/g) in cucumber plants infected with *S. sclerotiorum* under plastic house conditions.
Values represent means of 3 replicates. LSD (0.05): 7 days = 3.73, 14 days = 6.98, 21 days = 9.65.

CONCLUSION:

The study evaluated the effects of *Trichoderma aggressivum* and *Bacillus paralicheniformis* against the pathogen in vitro and under plastic house conditions. results showed strong antagonistic effects against *S. sclerotiorum* on cucumber, Earthworms (A. trapezoides) effectively consumed hydrated sclerotia, reducing the potential for soilborne infection. All treatments significantly reduced disease incidence and severity compared to the control. Enhanced peroxidase activity and phenolic content in treated plants indicate induced systemic resistance.

REFERENCES

[1] Al-Zaidi, A.K.N. and Al-Mharib, M.Z.K., 2025. RESPONSE OF GROWTH AND YIELD OF POTATO PLANTS TO ADDITION OF BIOFERTILIZERS, NILE FLOWER PEAT FERTILIZER AND SPRAYING WITH ITS EXTRACT. *Iraqi Journal of Agricultural Sciences*, *56*(1), pp.456-468. https://doi.org/10.36103/h499ej86

[2] Al, Matloob & et, A.Y. Abid, and K.Z. Khadhair. 2017. "Efficiency of Arbuscular Mycorrhizal Fungi and Some Species of Plant Growth Promoting Rhizobacteria to Control Fungi Fusarium Chlamydosporum Causing Agent of Decline Date Palm Off Shoots." *Iraqi Journal of Agricultural Sciences* 48(2). https://doi:10.36103/ijas.v48i2.418. https://doi.org/10.36103/ijas.v48i2.418

[3] Aljbouri, A.K. and Mahmood, S.M., 2023. EVALUTION THE EFFECT OF HEMAZOLE PESTICIDE ON THE VITALITY, GROWTH AND TISSUE CHANGES OF EARTHWORMS. *Iraqi Journal of Agricultural Sciences*, *54*(3), pp.667-673. https://doi.org/10.36103/ijas.v54i3.1744

[4] Amerio, NS, Barengo, MP, Bich, GA & Castrillo, ML 2024, 'Molecular interactions of *Trichoderma*: from microbial competition to soil health promotion', *International Journal of Molecular Biology: Open Access*, vol. 7(1) , pp. 135–138. https://doi.org/10.15406/ijmboa.2024.07.00183

[5] Anwar, Sh, F. Mahmood, N. A. Tahir, and G. F. Salih. 2022. "Secondary Compounds Released by Rhizospheric Bacteria Exhibit Fungistatic Effects Against Phytopathogenic Fungus." *Iraqi journal of agricultural sciences* 53(5): 1174–83. https://doi.org/10.36103/ijas.v53i5.1631

[6] Bell, D.K., Wells, H.D. and Markham, C.R., 1982. In vitro antagonism of Trichoderma species against six fungal plant pathogens. *Phytopathology*, 72(4), pp.379–382. https://doi.org/10.1094/phyto-72-379

[7] Buchwaldt, L., Garg, H., Puri, K.D., Durkin, J., Adam, J., Harrington, M., Liabeuf, D., Davies, A., Hegedus, D.D., Sharpe, A.G. and Gali, K.K., 2022. Sources of genomic diversity in the self-fertile plant pathogen, Sclerotinia

sclerotiorum, and consequences for resistance breeding. *PLoS One*, *17*(2), p.e0262891. https://doi.org/10.1371/journal.pone.0262891

[8] Chebotar, V.K., Gancheva, M.S., Keleinikova, O.V., Baganova, M.E., Zaplatkin, A.N., Pishchik, V.N. and Chizhevskaya, E.P., 2024. Whole-genome sequence of Bacillus paralicheniformis strain R243 isolated from rice (Oryza sativa L.) roots. *Microbiology Resource Announcements*, *13*(11), pp.e00879-24. https://doi.org/10.1128/mra.00879-24

[9] Cooke, B.M., Jones, D.G. and Kaye, B. 2006. The epidemiology of plant diseases (Vol. 2). Dordrecht, The Netherlands: *Springer*. p576. https://doi.org/10.1007/1-4020-4581-6

[10] Dixon, G.R. and Doodson, J.K. 1971. Assessment keys for some diseases of vegetable, fodder and herbage crops. *Journal National Institute of Agricultural Botany*, 12: 299–307. https://doi.org/10.1007/978-1-349-03704-9_11

[11] Elmer, W.H., 2009. Influence of earthworm activity on soil microbes and soilborne diseases of vegetables. *Plant Disease*, *93*(2), pp.175-179. https://doi.org/10.1094/pdis-93-2-0175

[12] Euteneuer, P., Wagentristl, H., Steinkellner, S., Scheibreithner, C. and Zaller, J.G., 2019. Earthworms affect decomposition of soil-borne plant pathogen Sclerotinia sclerotiorum in a cover crop field experiment. *Applied Soil Ecology*, *138*, pp.88-93. https://doi.org/10.1016/j.apsoil.2019.02.020

[13] Fagundes-Nacarath, I.R., Cavalcante, G.P., Brito, R.A.S., Costa, P.M.A., Debona, D., Maffia, L.A. and Rodrigues, F.Á., 2025. Antagonistic Potential of Different Species of Trichoderma Against Sclerotinia sclerotiorum. *Journal of Phytopathology*, *173*(1), p.e70012. https://doi.org/10.1111/jph.70012

[14] Félix-Gastélum, Rubén, Gabriel Herrera-Rodríguez, Norma Ávila-Alistac, and Elizabeth León. 2024. "Sclerotinia Sclerotiorum on Bean and Potato in Sinaloa: Etiology, Epidemiology and Alternatives for Management." *Revista Mexicana de Fitopatología, Mexican Journal of Phytopathology* 42(3). doi:10.18781/R.MEX.FIT.2404-6. https://doi.org/10.18781/r.mex.fit.2404-6

[15] Hammerschmidt, R., Nuckles, E.M. and Kuć, J., 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to Colletotrichum lagenarium. *Physiological plant pathology*, 20(1), pp.73-82. https://doi.org/10.1016/0048-4059(82)90025-x

[16] Hassan, A.K., 2013**.** The efficacy of some biological and chemical agents in inducing systemic acquired resistance against damping-off and root rot disease caused by Pythium spp. on pepper. PhD thesis, University of Baghdad, College of Agriculture, Department of Plant Protection.

[17] Hossain, M.M., Sultana, F., Li, W., Tran, L.S.P. and Mostofa, M.G., 2023. Sclerotinia sclerotiorum (Lib.) de Bary: Insights into the pathogenomic features of a global pathogen. *Cells*, *12*(7), p.1063. https://doi.org/10.3390/cells12071063

[18] Jan, N., Bhat, M.Y., Wani, A.H., Malik, M.A. and Jan, M., 2023. Incidence of white mould of bean and characterization of its causal pathogen, Sclerotinia sclerotiorum in Kashmir valley, India. *Archives of Phytopathology and Plant Protection*, *56*(8), pp.636-646. https://doi.org/10.1080/03235408.2023.2213396

[19] Jinal, H.N., Sakthivel, K. and Amaresan, N., 2020. Characterisation of antagonistic Bacillus paralicheniformis (strain EAL) by LC–MS, antimicrobial peptide genes, and ISR determinants. *Antonie Van Leeuwenhoek*, *113*, pp.1167-1177. https://doi.org/10.1007/s10482-020-01423-4

[20] K, Maysoon A., and Kareem U. Hassan. 2024. "Synergistic Effect of Bacillus Mucilaginosus and Pseudomonas Fluorescens on the Availability of Soil Potassium, Growth and Yield of Cucurbita Pepo L." *Iraqi Journal of Agricultural Sciences* 55(5): 1650–56. https://doi:10.36103/285hkz27. https://doi.org/10.36103/285hkz27

[21] Khallaf, H., Amasha, R., Samyah, S., Jastaniah, J. and Aly, M.**,** 2022. The antagonistic *Bacillus paralicheniformis* SHG3 for the biocontrol of some phytopathogens', *Ambient Science*,9(3),pp. 51-56. https://doi.org/10.21276/ambi.2022.09.3.ra02

[22] Kumar, S., Tiwari, R.K.S., Nirmalkar, V.K., Dayasagar & Thakur, Y., 2024. Ecological niches of Bacillus species and their multifaceted mechanisms in combatting plant pathogens. *Journal of Scientific Research and Reports*, 30(12), pp.1–10. https://doi.org/10.9734/jsrr/2024/v30i122696

[23] Lanoue, A., Burlat, V., Schurr, U. and Röse, U.S., 2010. Induced root-secreted phenolic compounds as a belowground plant defense. *Plant Signaling & Behavior*, *5*(8), pp.1037-1038. https://doi.org/10.4161/psb.5.8.12337

[24] Li, E., Zhu, N., Zhang, S., Xu, B., Liu, L. and Zhang, A., 2024. Efficacy of Trichoderma longibrachiatum SC5 Fermentation Filtrate in Inhibiting the Sclerotinia sclerotiorum Growth and Development in Sunflower. *International Journal of Molecular Sciences*, *26*(1), p.201. https://doi.org/10.3390/ijms26010201

[25] Manganiello, G., Nicastro, N., Caputo, M., Zaccardelli, M., Cardi, T. and Pane, C., 2021. Functional hyperspectral imaging by high-related vegetation indices to track the wide-spectrum Trichoderma biocontrol activity against soil-borne diseases of baby-leaf vegetables. *Frontiers in Plant Science*, *12*, p.630059. https://doi.org/10.3389/fpls.2021.630059

[26] Mckinney, H.H. 1923. Influence of soil temperature and moisture on infection of Wheat seedling by Helminthosporum sativum. *Journal of Agricultural Reseach,* 26: 195 – 217.

[27] Meena, R. K.; Patni, V. and Arora, D.K. 2008. Study on phenolic and Their Oxidative Enzyme in Capsicum annuum L. Infected With Geminivirus. *Asian Journal Experimental. Science*. 22(3):.310- 307.

[28] Olanrewaju, Oluwaseyi Samuel, Bernard R. Glick, and Olubukola Oluranti Babalola. 2017. "Mechanisms of Action of Plant Growth Promoting Bacteria." *World Journal of Microbiology and Biotechnology* 33(11): 197. https://doi.org/10.1007/s11274-017-2364-9

[29] Oyesola, O.L., Tonjock, R.K., Bello, A.O., Taiwo, O.S. and Obembe, O.O., 2024. Trichoderma: a review of its mechanisms of action in plant disease control. *Preprints*. https://doi.org/10.20944/preprints202405.1378.v1

[30] Puga-Freitas R, Barot S, Taconnat L, Renou J-P, Blouin M. 2012. Signal Molecules Mediate the Impact of the Earthworm *Aporrectodea caliginosa* on Growth, Development and Defence of the Plant *Arabidopsis thaliana*. PLoS ONE 7(12): e49504. https://doi.org/10.1371/journal.pone.0049504

[31] Rani, Ritu, Amrinder Kaur, Rohit Chhabra, and Sandeep Jain. 2024. "Role of Fungicides in Agriculture and Their Impact on Environment: A Review." *PlantArchives* 24(1). doi:10.51470/PLANTARCHIVES.2024.v24.no.1.139. https://doi.org/10.51470/plantarchives.2024.v24.no.1.139

[32] Sánchez-Montesinos, B., Diánez, F., Moreno-Gavira, A., Gea, F.J. and Santos, M., 2019. Plant growth promotion and biocontrol of Pythium ultimum by saline tolerant Trichoderma isolates under salinity stress. *International Journal of Environmental Research and Public Health*, *16*(11), p.2053. https://doi.org/10.3390/ijerph16112053

[33] Singh, S., Singh, A.K., Pradhan, B., Tripathi, S., Kumar, K.S., Chand, S., Rout, P.R. and Shahid, M.K., 2024. Harnessing Trichoderma Mycoparasitism as a Tool in the Management of Soil Dwelling Plant Pathogens. *Microbial Ecology*, *87*(1), pp.1-14. https://doi.org/10.1007/s00248-024-02472-2

[34] Singh, S.P., Keswani, C., Singh, S.P., Sansinenea, E. and Hoat, T.X., 2021. Trichoderma spp. mediated induction of systemic defense response in brinjal against Sclerotinia sclerotiorum. *Current Research in Microbial Sciences*, *2*, p.100051. https://doi.org/10.1016/j.crmicr.2021.100051

[35] Sofo, A., Khanghahi, M.Y., Curci, M., Reyes, F., Briones, M.J., Sarneel, J.M., Cardinale, D. and Crecchio, C., 2023. Earthworm-driven changes in soil chemico-physical properties, soil bacterial microbiota, tree/tea litter decomposition, and plant growth in a mesocosm experiment with two plant species. *Plants*, *12*(6), p.1216. https://doi.org/10.3390/plants12061216

[36] Valenzuela-Ruiz, V., 2024. Potenciales mecanismos de control biológico de *Bacillus paralicheniformis* TRQ65 contra hongos fitopatógenos. *Fitopatología*, *42*(4), p.44. https://doi.org/10.18781/r.mex.fit.2024-18

[37] Singh. R., Verghese. S. P., Bansal A. (2026). Silver Nanoparticles For Environmental Sustainability And Their Role In Biological Activities. International Journal of Engineering Sciences & Research Technology, 15(3), 1–8. ttps://doi.org/10.64149/j.ijesrt.15.3-1-8

[38] Yakkou, L., Raouane, M., EL Harti, A. and Amghar, S., 2022. Effect of earthworm's extracts (Aporrectodea molleri) on yeast growth: in vitro study. *Iraqi Journal of Agricultural Sciences*, *53*(1). https://doi.org/10.36103/ijas.v53i1.1522

[39] Compaore J. M., Zoma V., KaboreB., Kam S., (2026). Numerical Study of The Thermal Behavior of A Wall Made of Hollow Cement Blocks Containing Glass Powder. International Journal of Engineering Sciences & Research Technology, 15(5), 70-82. https://doi.org/10.64149/j.ijesrt.15.5.70-82

[40] Zhang, Y., Wang, Y., Wu, D., Qu, D., Sun, X. and Zhang, X., 2022. SNP and Haplotype Variability in the BnP5CR2 Gene and Association with Resistance and Susceptible Cultivars for Sclerotinia sclerotiorum in Brassica napus. *Agronomy*, 12(12), p.2956. https://doi.org/10.3390/agronomy12122956